Evaluation of Ki67 Expression in Relation to Tumor Stage and Fuhrman Nuclear Grade of Renal Cell Carcinoma and MUC1 Expression in Clear Cell Renal Cell Carcinoma

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ABSTRACT

BACKGROUND

Renal cell carcinoma (RCC) is the most common malignant renal tumour in adults. Prognosis of RCC depends on various factors like tumour stage, nuclear grade and histological type. For planning adjuvant therapy, accurate prediction of prognosis is mandatory. In many studies, ki67 and MUC1 has shown to be of prognostic significance and immunohistochemical expression of these two markers plays an important role in determining the prognosis of RCC. The purpose of this study was to evaluate the Ki67 expression in histologically confirmed cases of RCC and MUC1 expression in clear cell renal cell carcinoma, and to correlate them with the stage and Fuhrman nuclear grade of the tumour, in order to determine their role as prognostic markers in RCC.

METHODS

This study was a retrospective study. A total of 50 specimens of renal cell carcinoma were studied. The specimens were total and partial nephrectomy done in the department of urology for a period of 3 years. Expression of Ki67 and MUC1 in RCC were studied by immunohistochemistry (IHC). Statistical analysis was performed and P value < 0.05 was considered significant.

RESULTS

Out of 50 RCC studied, Ki67 labelling index ≥ 15 % was found in 35 cases. For MUC1, immunoreactivity of more than 10 % of tumor cell was found in 28/34 of clear cell RCC. In this study, Ki67 labelling index showed statistically significant expression with the stage of tumor and the nuclear grade. MUC1 expression also showed significant correlation with nuclear grade and stage of clear cell RCC.

CONCLUSIONS

High Ki67 labelling index in renal cell carcinoma is seen to correlate with higher nuclear grade and stage of tumor. High level expression with circumferential staining pattern of MUC1 is seen in high grade tumours with increased risk of metastasis. So MUC1 and Ki67 can be considered as a marker of prognosis of RCC.

KEY WORDS

Renal Cell Carcinoma, Immunohistochemistry, Ki67, MUC1

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BACKGROUND

Renal cell carcinoma is the most common malignant tumour of the kidney. It accounts for 2.5 % of all cancers[1] among the urinary system malignancy and RCC ranks third[2] after prostate and bladder tumour. Worldwide, RCC is the 9th most common malignancy in men and 14th in women. The case fatality rate was found to be low in highly developed countries than with low or medium level of socio-economic development.[3] Most of the renal cell carcinoma cases are sporadic and 2 % to 3 % are familial.[4] Symptoms of RCC includes the triad of hematuria, palpable abdominal mass and flank pain. Clear cell RCC is the most common type, representing 70 % of all RCCs, followed by papillary RCC which accounts for 10 % – 15 %, and then chromophobe RCC.[2] Different histological subtypes of RCC and tumours with the same grade and stage exhibit a wide variation in biological behaviour and prognosis. RCC is resistant to radiotherapy and chemotherapy. Nearly 40 % of RCC patients die because of disease progression.[5] At the time of diagnosis, approximately 30 % have metastasis and 20 % of patients develop metastasis even after curative treatment for localised disease.[6] Several clinical and pathologic factors affecting the prognosis have been reported in many studies. Prognostic factors are useful in determining the clinical and biological behaviour of RCC.[2] Patient presenting in the early stage of the disease were cured by surgery but those presenting with the metastasis are almost incurable.[4] Patients with poor prognosis should be identified early for timely initiation of target therapy. Study on molecular tumour markers expression can advance our understanding of the pathogenesis, diagnosis and treatment of RCC.[7]

Ki67 is a marker of active cell proliferation. Ki67 is absent in resting cells (G0 phase) and is present during all active phases of cell cycle (i.e. G1, S, G2 and mitosis[6]). In many studies, it has been reported that Ki67 expression predicts poor prognosis in solid tumours like breast cancer, cervical cancer, prostate cancer, glioma and hepatocellular carcinoma.[8] Proliferative activity of tumour cells can be determined by this marker. Ki67 was found to correlate with nuclear grade and it can be used for predicting the prognosis of RCC.

MUC1 is a large trans-membrane glycoprotein and is found on the apical surface of glandular cells and duct epithelial cells. In kidney, MUC1 was found on the luminal surface of distal tubule epithelium and collecting duct epithelium.[9] Tumor cells show loss of polarity and there is a switch from apical to circumferential staining. Aberrant de novo expression or over expression of MUC1 is supposed to play a role in the invasion and metastasis because of reduced cell adhesion.[10] In renal cell carcinoma, MUC1 is over expressed in clear cell RCC and its expression is found to be associated with the Fuhrman nuclear grade and tumour progression. Immunohistochemistry was performed with Ki67 and its expression was correlated with tumor stage and nuclear grade. MUC1 expression was also evaluated in clear cell RCC, with respect to its role in predicting the prognosis.

Objectives

The objective of this study was to study the clinicopathological features of renal cell carcinoma, to evaluate the Ki67 expression in renal cell carcinoma in relation to the histological types, tumor stage and nuclear grade, and to evaluate the MUC1 expression in clear cell RCC in relation to the tumor stage and nuclear grade.

Aim of this study was to evaluate the MUC1 and Ki67 expression as markers of prognosis in RCC.

METHODS

This study was a retrospective study. In our department, proportion of RCC cases among adult malignancies we received was 3 %. Sample size obtained using Danial's sample size formula was 45. RCC specimens received during this study period was 50 and all the specimens were included in the study. The specimens were total and partial nephrectomy done in the department of urology of tertiary care institute for a period of 3 years from January 2011 to January 2014.

Inclusion Criteria

All histologically confirmed renal cell carcinoma specimens according to world health organization (WHO) classification of tumours of the urinary system and male genital organs were included.[11]

Exclusion Criteria

1. Renal biopsy specimens
2. Renaltumours other than renal cell carcinoma

Ethical Approval

Permission of the Institutional Ethics Committee was obtained.

Data Collection

Clinical details such as age, gender, laterality, type of procedure done were collected for renal cell carcinoma specimens received during the period of study. Specimen were fixed in 10 % neutral buffered formalin. Grossing and reporting were done according to cancer protocol (CAP). Haematoxylin and eosin stained sections were prepared from paraffin tissue blocks. All the cases reported as renal cell carcinoma were selected. Parameters like histological type, Fuhrman nuclear grading, necrosis, sarcomatoid differentiation, capsular infiltration, ureter invasion, renal vessel invasion, lymph node involvement, distant metastasis and staging were performed. Histological subtype of renal cell carcinoma was done according to WHO classification of tumours of the urinary system and male genital organs.[11] Nuclear grading was done according to the Fuhrman nuclear grading system. Pathological staging was done according to American Joint Committee on cancer 2010.[12]

Immunohistochemical Staining for Ki67 and MUC1

Ki67 immunohistochemistry was done in all RCC cases and MUC1immunohistochemistry was performed on clear cell RCC cases. IHC was performed using monoclonal mouse clone

antibody-DAKO for detection of Ki67 and monoclonal rabbit antibody-PATHINSITU for detection of MUC1. Normal kidney with distal convoluted tubules and collecting ducts was taken as positive control for MUC1, and tonsil was taken as positive control for Ki67. Negative control was done by omitting the primary antibody.

Evaluation of IHC Staining

In Ki67 immunoreactivity, nucleus will be stained as dark brown in colour. Number of cells positive are counted and expressed as percentage. Cut off value was 15 % according to Mehdi MZ et al.[13]

A positive cell was considered, when MUC1 showed membranous, cytoplasmic or both staining patterns. Number of cells positive are counted and expressed in percentage. Immunoreactivity was graded as follows:

- no reactivity
- 1 - less than 10 % of cancer cells positive
- 2 - 10 – 25 % positive
- 3 - 25 – 50 % positive
- 4 – 50 – 75 % positive
- 5 - 75 – 90 % positive
- 6 - More than 90 % positive.

For statistical analysis, sample showing more than 10 % immunoreactivity was considered as positive.[9]

Statistical Analysis

The statistical analysis was performed using Statistical Package for Social Science (SPSS software version 15.5) which consisted computing the frequency counts and percentages for qualitative variables and mean for the quantitative variables. Chi square test was used to analyse the association between the different variables in the study and the P value below 0.05 was considered significant.

RESULTS

Totally 50 cases of renal cell carcinoma were studied with histopathology and immunohistochemistry. The cases were found in the age group of 28 - 80 years. The most common age group affected were 41 - 50 years. Males are more commonly affected and they account for 74 % of cases. Right kidney (68 %) was affected more commonly than left kidney. 60 % of patients had history of smoking. 61 % patients were hypertensive. Stage 1 was the most common stage at presentation and it accounts for 34 % of cases, followed by 26 % cases in stage 2, 26 % cases in stage 3 and 14 % cases in stage 4. 20 % of cases were nuclear grade 1, 28 % of cases were nuclear grade 2, 32 % of cases were nuclear grade 3 and 20 % of cases were nuclear grade 4 (Table 1).

Distribution of Renal Cell Carcinoma Based on Histological Types and Expression of Ki67 in Various Types

Among 50 cases of renal cell carcinoma studied, clear cell carcinoma was the most common type (68 %) followed by papillary RCC (16 %), chromophobe RCC (4 %) and unclassified type (12 %).For clear cell RCC, Ki67 labelling index was ≥ 15 % in 67.6 % of cases while the same is 62.5 % for papillary RCC and 50 % for chromophobe RCC (Figure 1); in unclassified type, all cases had labelling index ≥ 15 %. There was no significant association between Ki67 labelling index and histological type (P = 0.35).

		Frequency	Percentage %
Stage of tumor	Stage I	17	34
	Stage II	13	26
	Stage III	13	26
	Stage IV	7	14
Types of renal cell carcinoma	Clear cell RCC	34	68
	Papillary RCC	8	16
	Chromophobe RCC	2	4
	Unclassified	6	12
Fuhrman nuclear grading	Grade 1	10	20
	Grade 2	14	28
	Grade 3	16	32
	Grade 4	10	20

Table 1: Tumor Stage, Histological Types and Fuhrman Nuclear Grade of Renal Cell Carcinoma

		KI67 Labelling Index		Total	P Value
		< 15 %	➢ 15 %		
Type of RCC	Clear CELL RCC	11 (32.4%)	23 (67.6%)	34 (100%)	0.35
	Papillary RCC	3 (37.%5)	5 (62.5%)	8 (100%)	
	Chromophobe RCC	1 (50%)	1 (50%)	2 (100%)	
	Unclassified	0	6(100%)	6 (100%)	
Tumor stage	STAGE1	11 (64.7%)	6 (35.2%)	17 (100%)	0.003
	STAGE 2	4 (30.7%)	9 (69.2%)	13 (100%)	
	STAGE 3	0	13 (100%)	13 (100%)	
	STAGE 4	0	7 (100%)	7 (100%)	
Nuclear grade	GRADE 1	9 (90%)	1 (10%)	10 (100%)	<0.00001
	GRADE 2	6 (42.8%)	8 (57.2%)	14 (100%)	
	GRADE 3	0	16 (100%)	16 (100%)	
	GRADE 4	0	10 (100%)	10 (100%)	

Table 2: Ki67 Expression in Relation to the Histological Types, Tumor Stage, and Nuclear Grade of RCC

		MUC1 Grade							Total	P Value
		0	1	2	3	4	5	6		
Tumor stage	**Stage1**	2	4	2	3	0	0	1	12	0.006
	Stage 2	0	0	1	5	4	1	0	11	
	Stage 3	0	0	1	0	1	3	2	7	
	Stage 4	0	0	0	0	1	3	0	4	
Nuclear grade	Grade 1	2	3	3	1	0	0	0	9	0.0002
	Grade 2	0	1	1	6	2	0	0	10	
	Grade 3	0	0	0	1	1	7	1	10	
	Grade 4	0	0	0	0	1	2	2	5	

Table 3. MUC1 Expression in Relation to the Stage and Nuclear Grade of Clear Cell RCC

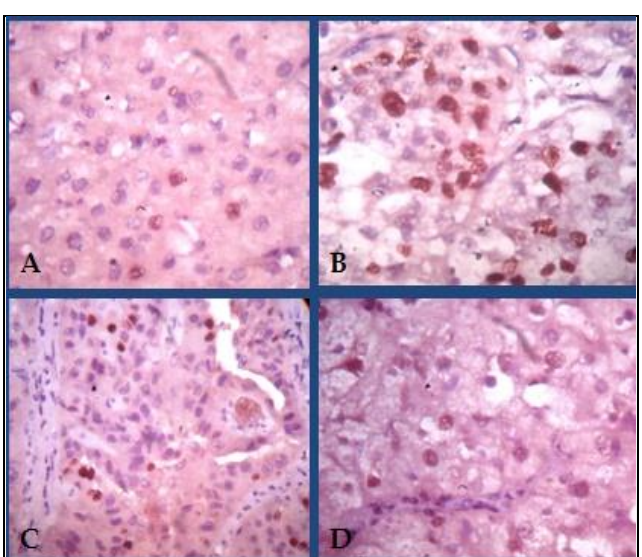

Figure 1. At 400x Magnification. Ki67 Expression in Low Grade Clear Cell RCC (A), Ki67 Expression in High Grade Clear Cell RCC (B), Ki67 Expression in Papillary RCC (C) and Ki67 Expression in Chromophobe RCC (D)

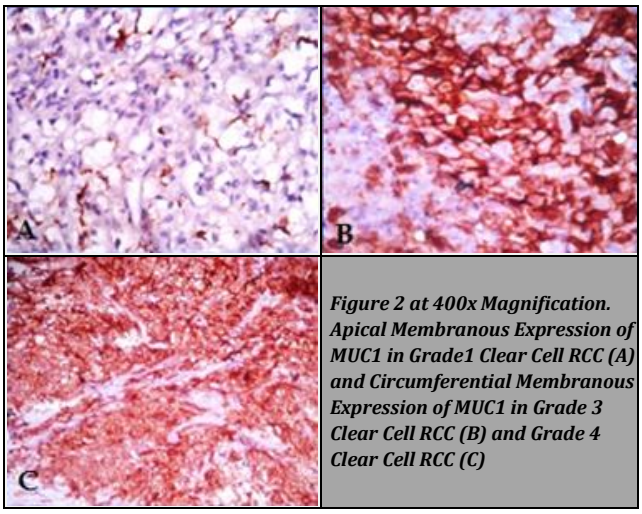

Figure 2 at 400x Magnification. Apical Membranous Expression of MUC1 in Grade1 Clear Cell RCC (A) and Circumferential Membranous Expression of MUC1 in Grade 3 Clear Cell RCC (B) and Grade 4 Clear Cell RCC (C)

Ki67 Labelling Index in Relation to the Stage and Grade of RCC

Out of 17 stage 1 tumours, 6 cases showed Ki67 labelling index ≥ 15 % and 11 cases showed Ki67 labelling index < 15 %. Out of 13 stage 2 tumours, 9 cases showed Ki67 labelling index ≥ 15 % and 4 cases showed Ki67 labelling index < 15 %. Among the stage 3 and stage 4 cases, all the cases showed Ki67 labelling index ≥ 15 %. So, Ki67 labelling index increases with increase in stage of tumour which was statistically significant (P value - 0.003).

Out of 10 grade 1 tumours, 1 case showed Ki67 labelling index ≥ 15 % and 9 cases showed Ki67 labelling index < 15 %. Out of 14 grade 2 tumours, 8 cases showed Ki67 labelling index ≥ 15 % and 6 cases showed Ki67 labelling index < 15 %. Among grade 3 and grade 4 tumours, all the cases showed Ki67 labelling index ≥ 15 %. So, Ki67 labelling index increases with increase in grade of tumour which was statistically significant (P < 0.00001) (Table 2).

MUC1 Expression in Relation to the Grade and Stage of Clear Cell RCC

MUC1 immunoreactivity of more than 10 % of tumour cells was found in 28/34 clear cell RCC. When staining pattern is considered, clear cell RCC showed apical or circumferential membranous staining pattern. Among the clear cell RCC, stage of tumour (P - 0.006) and nuclear grade (P - 0.0002) showed statistically significant MUC1expression (Table 3). In clear cell RCC, apical membranous expression was found in low nuclear grade tumours (grade 1 & 2) and low stage tumours (stage 1 & 2) whereas circumferential expression was found in high nuclear grade tumours (grade 3 & 4) and high stage tumours (stage 3 & 4) (Figure 2).

DISCUSSION

Renal cell carcinoma is the most common urological malignancy with an unpredictable clinical course[2].Among the urological malignancies, it has the poorest prognosis.[13] Prognosis of tumours with same histological type and stage varies between patients to patient. In RCC, nuclear grade and tumour stage are the two important factors that can predict the prognosis of tumour. The most commonly followed nuclear grading system was Fuhrman grading system. This system often shows inter observer and intra observer variation.[14] With the advent of targeted therapies, many studies were performed with immunohistochemistry markers to predict the prognosis, for treatment planning and follow up.

Ki67 is a marker of active cell proliferation. In many studies, there was a significant association between the Ki67 index and RCC patient`s survival.[7] In our study, Ki67 labelling index ≥ 15 % is seen in 70 % of cases and this was correlating with the study done by Amouian et al.[15] among 30 cases with 66.6 % positive for Ki67. The positive expression rate of Ki67 was 47.7 % in the study done by Kaizheng et al.[16] which included 1239 cases. 53.7 % expression was found in the study done by SezenKocarsian[17] which include 67 cases of RCC.

In our study, Ki67 expression was in correlation with nuclear grade and this was similar to the study done by Muhammad Zain Mehdi,[13] Matthew H. T Bui et al.[7] Kaizheng et al.[16] and Amouian et al.[15] In our study, Ki67 expression has significant correlation with stage of tumour and this was similar to the study done by Kaizheng et al.[16] Onda H et al.[18] and Bidisha Chakraborty et al.[3] There was no significant correlation between Ki67 expression and histological subtypes.

MUC1 is a trans membrane mucin protein which belongs to the human mucin family expressed by the epithelial cells. In case of its abnormal expression, there will be circumferential type expression in tumour cells and such expression pattern is suspected to destabilise cell to cell adhesion, thereby favouring metastasis. In our study, MUC1 immunoreactivity of more than 10 % of tumour cells was found in 28/34 clear cell RCC. Among the clear cell RCC in our study, nuclear grade of tumour showed statistically significant MUC1 expression (P = 0.0002), with low grade tumours showing apical membranous type expression and high grade tumours showing circumferential membranous type expression. Among the clear cell RCC in our study, stage of tumour r also showed statistically significant MUC1 expression (P = 0.006), with low stage tumours showing apical membranous type expression and high stage tumours showing circumferential membranous type expression. This was correlating with the study done by Cord Langer et al.[9] They found statistically significant MUC1 expression with tumour stage (P - 0.002) and nuclear grade (P - 0.0001), when staining pattern is considered, with low stage and low grade tumours showing predominantly apical membranous staining pattern whereas high stage and high grade tumours showing predominantly circumferential membranous expression. Similarly, MUC1 expression showed statistically significant correlation with nuclear grade (P < 0.005) among 44 pT1Clear cell RCC cases in the study done by Xavier Leroyet al.[19] Again, in the study done by Fujita et al.[20] among 51 cases of clear cell RCC, they observed that the low grade tumours show less MUC1 staining than high grade tumours and metastatic tumours show more MUC1 staining than the localised tumours.

In our study, MUC1 expression was studied only in clear cell RCC and so more studies with other histological types should be carried out to evaluate correlation of nuclear grade

and stage with MUC1 expression in other histological types of RCC.

CONCLUSIONS

High Ki67 labelling index in renal cell carcinoma is seen to correlate with higher nuclear grade and stage of tumour. High level expression with circumferential staining pattern of MUC1 is seen in high grade tumours with increased risk of metastasis. So MUC1 and Ki67 can be considered as a marker of prognosis of RCC. Further, large studies with multivariate analysis should be done to demonstrate MUC1 as a prognostic marker and as a therapeutic target for RCC.

Data sharing statement provided by the authors is available with the full text of this article at jemds.com.
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